SUMMARY OF THE U.S. EPA COLLOQUIUM ON A FRAMEWORK FOR HUMAN HEALTH RISK ASSESSMENT

Colloquium #1

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SECTION ONE

BACKGROUND

Developing a Framework for Human Health Risk Assessment

The U.S. Environmental Protection Agency (EPA) has recognized the need to develop a framework for human health risk assessment that puts a perspective on the approaches in practice throughout the Agency. Current human health risk assessment approaches are largely endpoint driven. In its 1994 report entitled *Science and Judgment in Risk Assessment*, the National Research Council (NRC) noted the importance of an approach that is less fragmented, more consistent in application of similar concepts, and more holistic than endpoint-specific guidelines. Both the NRC and EPA's Science Advisory Board have raised a number of issues for both cancer and noncancer risk assessment that should be reconsidered in light of recent scientific progress. EPA has recognized the need to develop a more integrated approach. In response, the Agency's Risk Assessment Forum (RAF) has begun the long-term process of developing a framework for human health risk assessment.

The framework will be a communication piece that will lay out the scientific basis, principles, and policy choices underlying past and current risk assessment approaches and will provide recommendations for integrating/harmonizing risk assessment methodologies for all human health endpoints.

As an initial step in this process, the RAF formed a technical panel in April 1996. An Issues Group (Gary Kimmel and Vanessa Vu, co-chairs; Jane Caldwell; Richard Hill; and Ed Ohanian) was formed, and this group developed a white paper, entitled *Human Health Risk Assessment: Current Approaches and Future Directions*, to provide an overall perspective on the issue (see Appendix A). The RAF peer-reviewed the white paper in February 1997. Its purpose is to serve as a basis for further discussion on current and potential future risk assessment approaches. The paper highlights a number of issues regarding the Agency's risk assessment approaches and their scientific basis, primarily with respect to dose-response and hazard assessment. The paper discusses the scientific basis for cancer and noncancer risk assessment, including differences and similarities. It also identifies knowledge/information gaps and areas where more work is needed.

As part of the continuing effort to develop a human health risk assessment framework, the RAF organized a colloquium series, consisting of two internal colloquia. The colloquia are intended to bring together EPA scientists for a dialogue on various scientific and policy issues pertaining to EPA's cancer and noncancer risk assessment approaches. The first colloquium, held on September 28 and 29, 1997, in Arlington, Virginia, focussed on the role of mode of action information in re-examining and developing new risk assessment approaches. The second colloquium, to be held in early 1998, will be more quantitative in nature and will focus on dose-response considerations, including low-dose extrapolation methods.

The overall goal of the first two colloquia is to provide Agency scientists an opportunity to share perspectives on the role of mode of action in shaping future human health risk assessment approaches. As the Agency moves forward to develop this framework, additional colloquia are anticipated, as well as workshops to gather input and perspectives from scientists outside EPA.

The September 1997 Colloquium

The RAF invited a cross-section of senior Agency scientists (from headquarters, Research Triangle Park, and the regions) to participate in round table discussions (see participant list in Appendix B). Participants engaged in active and open discussions throughout the 2-day colloquium, both in plenary and breakout sessions. The group discussed the current standard default approach for cancer and noncancer risk assessment, and the advantages and limitations of departing from this approach in light of new information pertaining to chemical mode of action. The primary topics deliberated by the group included defining mode of action, evaluating what events are critical, determining when enough information exists to support new risk assessment approaches, and strategizing on how mode of action information can be effectively and systematically used in low-dose extrapolations.

Prior to the first colloquium, each participant received the white paper, five case studies (Appendix C), a "charge" (Appendix D), a working definition of "mode of action," and a list of questions developed to guide colloquium discussions (Appendix E). An outside speaker, Rory Conolly from the Chemical Industry Institute of Toxicology (CIIT), was invited to open the colloquium. His presentation, like the white paper, was intended to elicit thought and help initiate group discussion on past, current, and potential future risk assessment approaches.

Melvin Andersen, ICF Kaiser Inc., K.S. Crump Group, served as the colloquium facilitator. He presented an overview of the case studies and throughout the colloquium guided group discussions to ensure that the general and specific questions were deliberated. Each participant was assigned to one of three breakout groups (see group assignments in Appendix F). In making the group assignments, EPA sought to ensure a mix of expertise and Agency representation in each group. A group leader helped to facilitate discussions in each breakout group and a rapporteur captured key discussion points and group consensus.

The colloquium was structured as a series of alternating plenary sessions and small group discussions (see Agenda, Appendix G). Initial group discussions addressed general risk assessment issues and the overall use of mode of action in risk assessment. Case study discussions followed. The colloquium's final session included full group discussions on "critical harmonization issues" and quantitative dose-response issues to be covered at the next colloquium.

SECTION TWO

OPENING PLENARY SESSION

INTRODUCTORY PRESENTATIONS

Welcoming Remarks

To open the colloquium series, William Wood, Director of the Risk Assessment Forum, welcomed all participants and observers. He thanked all those who helped in developing the colloquium series, including members of the planning committee[1] and authors of the white paper. He emphasized the primary goal of the colloquium series: to provide an opportunity for Agency staff to exchange perspectives on mode of action and harmonization issues in human health risk assessment. He commented that the response to the colloquium series was very positive, noting that at least half of EPA's risk assessment community was represented at this event. The overall expectation is that participants will come away with a general appreciation for the use of mode of action, its limitations, challenges, and utility in the risk assessment arena.

Background/Goals of the Human Health Risk Assessment Framework

Vanessa Vu of EPA's Office of Pollution Prevention and Toxics, and co-chair of the planning committee, provided some background on the colloquium series and an overview of the goals of the human health risk assessment framework (see Section One). Dr. Vu emphasized that, because of evolving science, EPA has been challenged to use mechanistic information in risk assessment instead of the current endpoint-specific approach. The following questions/issues, therefore, need to be examined in light of the newer science:

- Is routine application of nonthreshold and threshold approaches for cancer and noncancer endpoints, respectively, appropriate in all cases?

- How should EPA treat dose-response for the observable range (e.g., benchmark dose, point estimate, 95% lower confidence limit, etc.)?

- How should doses be adjusted across species?

- How should less than lifetime exposures be evaluated?

Dr. Vu explained that the scope of Colloquium #1 was to discuss qualitative aspects of mode of action, with the case studies serving to help focus discussions. Because of the limited time available for breakout group discussion, the five case studies could not include all possible areas of interest (e.g., portal of entry considerations, mode of action of certain endpoints), but instead were designed to serve as a stepping off point for discussions on mode of action and the harmonization of human health risk assessment approaches.

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[1]Planning committee members include Vanessa Vu (co-chair), Gary Kimmel (co-chair), Bill Wood, Kim Hoang, Annie Jarabek, Jennifer Seed, and Wendy Yap.

Keynote Speaker

Rory Conolly, a Senior Scientist at CIIT, provided the group with his views on risk assessment approaches, speaking about the relevance of mode of action and dose-response modeling in shaping future risk assessments. The central question he addressed in his presentation, entitled "Evolution of Human Health Risk Assessment: Using Biological Information to Define Modes of Action, Develop Exposure-Response Models, and Refine Default Assumptions," was, How do we move forward and bring the newer science into risk assessment at a reasonable and responsible rate? Highlights of the presentation are provided below; a copy of the speaker handout is presented in Appendix H.

Historical Perspective

In the 1970s, only a limited understanding of mechanism of action existed. Default methods and models were based on state of the science at that time and were therefore appropriate.

In deriving risk assessment methodologies, regulators have strived to minimize uncertainty and derive reasonable risk estimates, balancing the desire not to miss any risks with the desire not to overestimate risk and incur unnecessary compliance costs. Looking to the future, risk assessors should continue to seek to reduce uncertainty in risk assessment, using mechanistic data where possible to improve predictions of risk.

Where Are We Today?

Today we have a larger data base and a better understanding of mode of action in cancer and noncancer response. It is, therefore, appropriate to use the latest science and to update risk assessment practices.

A lag time between availability of new science and acceptance and use in practice is inevitable. Moving forward requires reaching consensus, which involves working out the details and developing methodology to use the new science.

How to Get Science Into Risk Assessments

As understanding improves, risk assessment policies need to be re-evaluated; EPA's new cancer guidelines show how the Agency is starting to do this.

More sophisticated validated models (PBPK and biologically based) for dose-response need to be developed. Ideally, we need models to describe the whole exposure response process. Exposure-response models are available but are not as well developed as PBPK models; they have been used for dioxin, 5-fluorouracil, chloroform, and formaldehyde.

When are models mature enough for widespread use? To be used in risk assessment, a model needs to be validated against animal and human data; receive adequate quality control; and be peer-accepted.

Challenges for Regulators/Where Is Risk Assessment Going?

Regulators face the challenge of incorporating evolving and more sophisticated approaches into risk assessment methodology. Guidelines need to be developed to identify acceptable models for use in risk assessment; criteria can be qualitative (e.g., taking component parts of a model, comparing it to the default approach, and deciding whether uncertainty is increasing or decreasing).

Evaluation of mechanistic data will not be easy. A wide spectrum of interactions/mechanisms exist; some do not result in toxic effects. Therefore, substantive questions exist concerning how one evaluates various biomarkers and relates them to toxicity.

Additional data are needed to fully understand the shape of the dose-response curve at low levels of exposure and to effectively incorporate these data into the quantitative risk assessment. Experimental work could be performed to address this knowledge gap.

Computer models have become cheaper, faster, and increasingly sophisticated. We can now incorporate biology into models (e.g., models showing airflow through the nasal passages of rats and humans)—something that could not be done 10 years ago. The nasal passage models demonstrate that detailed anatomical modeling can make a difference in risk predictions; this is a lesson for any organ with anatomical complexity—we should continue to develop such models and use them in risk assessment.

Defaults will continue to be important in risk assessment, but we need to keep up with the science. Some of yesterday's defaults will not be good enough for tomorrow. Most chemicals will not have rich data sets (may have limited but targeted data collection [e.g., PBPK models, short-term assays, predictive computer model]). Defaults will still need to

be used in the future, but they will be enriched by the newer science and modeling technologies.

Well-articulated risk assessment strategy can motivate research, specifically in terms of how biologically based modeling is incorporated into risk assessment. EPA could take the lead in specifying data needs (e.g., the kind of descriptive data needed, the criteria needed for validation of models, and the role human models should play in model validation). Promulgation of new risk assessment guidance using mechanistic data is needed to encourage industry to pay for research. Industry needs to be sensitive to the fact that some lag time is inevitable, but regulators should not let the lag time get too long.

Introduction to Case Studies and Colloquium Issues and Charge to the Breakout Groups

Melvin Andersen of ICF Kaiser, Inc. facilitated the colloquium. In his introductory remarks he encouraged the group to engage in active discussions on how to use mode of action information wisely. To open discussions, Dr. Andersen reviewed the definition of mode of action developed for the purposes of this colloquium.

> *Mode of action is defined as those key biological events that are directly linked to the occurrence of toxic responses. These events include absorption and entry into the body up to the final manifestation of toxicity.*

Dr. Andersen suggested that the group keep the following questions/issues in mind when thinking about mode of action.

What is the nature of the chemical causing the effect?

What are the initial interactions that a chemical has with macromolecules or cellular components?

All details may not be necessary, but the challenge lies in deciding on how to incorporate available new information.

Information on what is happening at the molecular level will continue to grow. New guidance emphasizes mode of action (e.g., IARC, NRC, EPA). Our choices are either to continue to be proactive or be reactive later.

Dr. Andersen charged the group to begin exchanging ideas and perspectives in the first breakout session, specifically discussing the definition of mode of action and general questions pertaining to mode of action and risk assessment (see Section Three).

Dr. Andersen then provided the group with a brief overview of the case studies, explaining that the case studies were developed to emphasize diverse issues and that the nine general

questions (see Section Four) provided to participants were intended to guide discussions. The first four questions are somewhat generic in nature, while the remaining questions focus more on mode of action and how information can be used to influence decisions on risk assessment approaches.

Questions/Comments

As summarized below, a brief group discussion followed the keynote address and introductory remarks by EPA and the facilitator.

One attendee questioned how feasible it might be to apply work done in the pharmaceutical industry (where a significant amount of human data and mode of action information are available) to developing PBPK models and validating existing animal models for the chemicals of interest to EPA, FDA, etc. Responses indicated that while some of this information is available for the therapeutic effects of anticancer drugs and has been used in the development of fundamental pharmacokinetic models, data are largely unavailable for the toxic effects of those drugs. The goal of most pharmacokinetic studies in the pharmaceutical industry is to get information on the therapeutic dose range, not to learn specifically how the chemical acts. PBPK models for pharmaceutical drugs have not been widely used for the type of extrapolations used by EPA in studying toxic chemicals (e.g., species or high to low dose extrapolations). The industry is beginning to see biologically based models as a good adjunct to human data. Such models may be used by the industry to evaluate developmental/reproductive effects where little human data are available. A few participants noted that acquiring any available data may be difficult because of confidentiality issues and the existence of a great deal of chemical-blind data.

Another participant commented that we may never have low-dose information for the endpoints EPA is currently studying, but emphasized the importance of starting to study mechanistic effects in the low-dose range and linking those events to the observed effect of regulatory interest.

SECTION THREE

BREAKOUT GROUP DISCUSSIONS ON GENERAL QUESTIONS

The opening plenary session was followed by a breakout session designed to give the colloquium participants an opportunity to open discussions on the role of mode of action in risk assessment. The three breakout groups were charged with discussing the following three questions:

Question #1	What are the variety of different purposes for which EPA conducts risk assessments?
Question #2	How has mode of action information been used in risk assessment to date?
Question #3	Are there differences in the importance of mode of action information for conducting risk assessments for different human health endpoints/toxicities?

When participants reconvened in plenary session, Vicki Dellarco, Carole Braverman, and Mark Stanton summarized the discussions from Breakout Groups 1, 2, and 3, as presented below.

What Are the Variety of Different Purposes for Which EPA Conducts Risk Assessments?

The breakout groups identified multiple ways in which EPA uses risk assessment. With the protection of human health and the environment stated as the primary purpose, the groups identified the following specific examples of why and when EPA conducts risk assessments:

- Setting regulatory standards
- Educational purposes
- Screening level analyses
- Measuring public health impact
- Determining important toxic endpoints
- Identifying susceptible receptors
- Evaluating site remediation options
- New product and pesticide registration/cancellation
- Setting acceptable exposure levels
- Evaluating the need for emergency actions
- Evaluating residual risk
- Deriving drinking water standards
- Permitting
- Supporting state regulations
- Coperating with the international community
- Evaluating pollution prevention approaches
- Setting ambient water quality criteria
- Responding to public and Congressional requests
- Reporting toxic release inventory
- Ranking/prioritizing chemicals
- Identifying data needs
- Setting priorities for research

How Has Mode of Action Information Been Used in Risk Assessment to Date?

The groups identified the following ways in which mode of action information has been used to date in the risk assessment process.

- PBPK modeling.
- Distinguishing between noncancer and cancer effects (threshold versus nonthreshold).
- Identifying endpoints by looking at precursor events.
- Identifying the hazard expression, emphasizing early life risk (e.g., vinyl chloride).
- Evaluating species differences and the relevance of animal data to humans (e.g., 1,3-butadiene, alpha-2μ globulin).
- Integrating data, evaluating anatomical precursor events (e.g., ozone).
- Identifying endpoints of concern by looking at important precursor events (e.g., vinyl chloride, butadiene).
- Grouping compounds by a common mechanism of toxicity (e.g., antithyroid compounds or cholinesterase inhibitors).
- Strengthening the basis for certain hazard calls and the justification for certain quantitative approaches (e.g., melamine—strayed from low-dose linear approach).
- Influencing the endpoint used to choose an RfC/RfD (e.g., vinclozaline as an anti-androgen).
- Excluding a particular animal model because it does not capture human risk.
- Adding risks for common mode of action (e.g., noncancer effects)

Are There Differences in the Importance of Mode of Action Information for Conducting Risk Assessments for Different Human Health Endpoints/Toxicities?

The consensus reached on this question was that no differences exist; mechanistic information is applicable to both cancer and noncancer assessments. Mode of action is important in both cancer and noncancer risk assessment and we therefore need to consider biology and route differences for all endpoints.

During the discussion of the general questions, several points were made regarding the utility and limitations of mode of action in risk assessment:

- # While there has been a philosophical desire to use mode of action in risk assessment, its use has been limited by the lack of available data and time. Historically, mode of action has generally been used more qualitatively in hazard identification versus quantitatively in dose-response assessment. Interest in looking more closely at mode of action exists; linking the limited amount of information to the endpoint will be the challenge.

- # At an international meeting held in the summer of 1997 to discuss mechanistic data in risk assessment, a number of participants reportedly were not fully convinced that using mode of action data would allow for better risk assessments.

- # Interspecies differences do not appear to get the attention of regulators (i.e., using mechanistic data in animals to predict human toxicity).

- # With limited resources in the regions, risk assessors/managers may not be able to consider comprehensive chemical-specific toxicity evaluations. While the benefit of this type of exercise is recognized by the regions, regional staff still look for numbers/bottom lines. In addition, from a regional perspective, improving fate and transport understanding is equally important to improving toxicity assessment.

- # Several participants noted that scientists are developing new gene tests and building data banks, but may not necessarily have risk assessment in mind. It is not clear exactly how this information may be used or integrated into regulations or guidelines.

- # It is hoped that, within the next decade or so, toxicity tests will be designed to provide both a less expensive and more informative test system (with mode of action in mind). Scientists and risk managers will need to look at new science and develop a process to use the data. Participant input at these colloquia will help shape that process.

- # Overall, participants recognized the advantages of using mode of action, but acknowledged that the process of systemizing the information will be difficult. A clearer definition of how mode of action will/can be used is needed. To date, mode of action has been used on a case-by-case basis, but a growing body of data is being developed from which we can learn and shape future efforts. No unifying systematic approach exists now, however. In developing existing toxicity values (e.g., RfDs, slope factors), numerous chemicals were studied; going back and re-examining mode of action for all of these chemicals is a daunting task; a way to streamline research efforts is therefore critical.

- # Participants recognized that as the process of evaluating the role of mode of action in risk assessment continues, caution must be taken not to fall into the “paralysis by analysis” trap. Several participants emphasized the importance of having a process in place so that

scientists do not become bogged down with too much information. Understanding every molecular event is not necessary to make a decision on activity (e.g., we do not understand all events in mutagenicity, but we still consider it a precursor to cancer).

- The group raised the following questions: 1) Will mode of action evaluation result in more confusion? 2) When will mode of action be ready for "prime time?" 3) When will the scientific community be ready to accept the process?

- The recently enacted Food Quality Protection Act mandates that a screening process for estrogens and other hormonally active mechanisms be established. Data will be collected for thousands of chemicals; the screening will look for estrogen or anti-androgen action in these chemicals. This example illustrates how mode of action considerations have helped determine endpoints.

- Participants again emphasized that working through new approaches does not mean past approaches are being criticized; EPA is merely trying to evaluate how new data sets can be used to improve human health risk assessment.

SECTION FOUR

BREAKOUT GROUP DISCUSSIONS ON CASE STUDIES

More than half of the 2-day colloquium was devoted to discussing the five case studies (see Appendix C). The case studies, "loosely designed" after real chemicals, were developed to help foster group discussions on qualitative issues critical to re-evaluating risk assessment approaches, such as selecting endpoints of interest, considering the influence of mode of action, identifying common critical events, and evaluating whether a data set supports using an alternative approach to the default dose-response analysis. Each case study included five sections: 1) a brief introductory section highlighting general compound properties/characteristics; 2) toxicokinetics; 3) effects in humans; 4) effects in animals; and 5) additional data relevant to mode of action.

Case study discussions focussed on the questions listed below. Participants also were encouraged to consider the broader questions of where mode of action and harmonized approaches were most evident.

- What are the toxic effects associated with the compound?
- How similar are the effects in studies of animals and humans?
- How consistent are the data across species and routes of exposure?
- At what administered doses or exposure concentrations are the effects observed?
- What do we know about mode of action for the different toxicities?
- Is mode of action influenced by dose (i.e., administered dose or exposure concentration)?
- Are there commonalities in mode of action for the various toxicities?
- Do we have enough information to determine a common critical event that leads to all subsequent toxicities for the compound? Is such a common precursor effect expected as a general rule?
- Qualitatively, how does mode of action information influence decisions about choice of risk assessment models for the dose-response analysis?

All three breakout groups reviewed and discussed Case Study A and Case Study B, both in individual breakout sessions and in plenary sessions. Each of the three breakout groups also examined and discussed one of the remaining case studies (i.e., Case Studies C, D, and E).

Breakout session discussions were open and lively, with active participation by all group members. While in some cases participants expressed frustration with the task of sorting through sometimes limited case study data, the exercise served its purpose in fostering discussions on the role of mode of action in the risk assessment process. The groups worked through the case studies, formulating hypotheses, where possible, regarding mode of action and evaluating whether any consistency existed across endpoints. (Some participants noted that the information in most of the case studies only enabled limited discussions on harmonization across endpoints.)

The case studies served to highlight the challenges and limitations of working with available data sets, sometimes making mode of action and harmonization decisions difficult. The groups explored additional data needs, focussing on data that would help support a decision on the appropriate low-dose model to be used. Some of the requested data will be important for the second colloquium, where the group will examine quantitative issues and low-dose extrapolation models.

The sections below summarize the main points discussed during the breakout sessions, captured by the group rapporteurs, and presented in the plenary sessions. Vicki Dellarco, James Rowe, and Mark Stanton presented Case Study A for Groups 1, 2, and 3, respectively. For Case Study B, Vicki Dellarco, Jane Caldwell, and Rita Schoeny presented for Groups 1, 2, and 3, respectively. Annie Jarabek, Oscar Hernandez, and Mark Stanton presented breakout group findings for Case Studies C, D, and E, respectively. Other group members contributed to the presentations and subsequent discussions on an ad hoc basis. The group presentations for Case Studies A and B were structured more strictly around the general questions listed above. While the presentations for the last three case studies captured the essence of the general questions, the presentations focussed more on summarizing the study and presenting mode of action and model hypotheses.

Case Study A

Compound A, as described in the case study, is a relatively stable, low molecular weight halogenated compound. It is used as a solvent and is a common byproduct of chlorination. Compound A is readily absorbed via inhalation and oral exposures and requires enzymatic catalysis in the body. The case study focusses on the toxic and carcinogenic actions on the nasal passage, kidney, and liver in chronically exposed animals.

Case Study A proved to be the most difficult case study, largely because it was the first, but also because of the nature of Compound A and the multiple issues associated with it. Participants identified additional information that would be needed before they could seriously consider nondefault approaches. General points and responses to the case study questions are presented below.

- *Toxic Effects*: Toxic effects associated with Compound A include nasal toxicity; cancer and noncancer effects in the liver and kidney; central nervous system depression; and cardiac arrhythmias.

- *Differences Between Animals and Humans*: Human data are limited. Effects appear to be similar in the liver in animals and humans.

- *Consistency Across Species/Exposure Routes*: Effects are similar in the liver.

- *Administered/Exposure Dose at Which Effect Is Observed*: Insufficient data are available for humans. Strong dose relationship observed in animals, but difficult to extrapolate to low doses.

- *Mode of Action for Different Toxicities*: Response is observed primarily in high metabolic tissues (i.e., the liver, kidney, and nasal passage) where high localized patterns of P450 are observed. Kidney response is related to cell proliferation. The data suggest that the action of Compound A is systemic, based on the results of the gavage and drinking water studies, but the data do not clearly support a commonality in mode of action across endpoints.

  Mode of action hypotheses presented by the breakout groups included 1) glutathione depletion resulting in cytotoxic response; and 2) oxidative metabolism, forming the unstable ketohalogen.

- *Influence of Dose on Mode of Action*: Differences seen in effects resulting from corn oil versus drinking water are a function of dose, not route of exposure. Dose appears to affect oxidative metabolism, but not glutathione depletion.

- *Common Critical Event for All Toxicities*: Based on available data, the group could not reach a consensus on a common critical event, although the requirement for metabolic transformation for all effects was noted.

- *Influence of Mode of Action on Risk Assessment Dose Model*: Based on the information presented in the case study, the group concurred that the default linear approach should be used for cancer effects. Because tumor development was determined to be secondary to cytotoxicity, the use of the margin of exposure (MOE) approach was suggested; multiple models, however, would need to be used (perhaps different models for different dose ranges). The group agreed that additional data are needed to support decisions regarding linear and MOE approaches.

- *Additional Data Needs*: The groups identified data gaps that limited the ability to answer certain questions. Suggested data needs include study design information; time-dependency data on cell proliferation; documentation of experimental dose levels for all

key studies; information on all target organs (or an indication that information for certain target organs is not available); additional human data, including clinical observations and molecular epidemiology data (e.g., changes in genes/biomarkers); and data to better evaluate site concordance.

Participants tended to fall back on the cancer/noncancer default approaches in the absence of certain data. More discussion of noncancer effects was recommended.

Case Study B

Compound B, widely used as an intermediate in chemical synthesis, dissolves easily in water, and is a gas under ambient conditions. Inhalation of Compound B is considered the most important route of exposure. The case study focussed on the cancer and reproductive/developmental effects associated with Compound B.

Compound B is well absorbed, but reacts at the exposure site. It is very reactive and alkylates critical macromolecules. Its metabolism leads to decreased activity. Genotoxic data show mutagenicity *in vitro* and *in vivo*, formation of DNA adducts, and an increase in micronuclei and sister chromatid exchange. Both cancer and reproductive/developmental effects are observed. All groups agreed that a common mechanism across endpoints was evident (i.e., alkylation of DNA) and that the low-dose extrapolation (based on genetic effects) should be similar for all endpoints, although different dose metrics may be needed to assess cancer versus developmental/reproductive outcomes because relatively short exposures may elicit developmental/reproductive effects.

The facts that support the groups' conclusions are summarized below. The groups presented a fairly long "wish list" (see additional data needs section below) but expressed mixed opinions regarding the extent of additional data needed to support a harmonized approach and develop a low-dose model for Compound B.

- *Toxic Effects*: Toxic effects associated with Compound B include cancer, reproductive effects, and other noncancer effects such as eye irritation, nausea, headache, and memory loss.

- *Differences Between Animals and Humans*: Cancer and reproductive effects are observed in humans, rats, and mice, but human data are limited. Irritant, respiratory, and neurological effects have been reported in humans. Developmental effects have been reported in rats and mice.

- *Consistency Across Species/Exposure Routes*: Groups noted that relatively consistent data exist for rodent species, although sex differences were difficult to discern from the case study data set.

- *Administered/Exposure Dose at Which Effect Is Observed*: There is a need for further evaluation of different dose metrics for cancer versus developmental effects. Short exposures may elicit reproductive/developmental effects. Exposure duration issues need to be explored more fully.

- *Mode of Action for Different Toxicities*: Effects are due to genetic damage by alkylating agents; there is a common mechanism, but a variety of targets. A brief discussion was held on the possibility of reaction with proteins/enzymes versus DNA; the consensus was that the focus should be on the overall mechanism (also considering repair mechanisms), not on the action on a single enzyme.

- *Influence of Dose on Mode of Action*: The groups could not fully evaluate dose issues based on the available data set.

- *Common Critical Event for All Toxicities*: Evidence suggests a common mode of action, but additional information is needed to support this hypothesis (see below).

- *Influence of Mode of Action on Risk Assessment Dose Model*: The groups concurred that a low-dose linear model is appropriate for cancer endpoints (parent compound at target site), although some participants were reluctant to assume linearity at low doses. For reproductive endpoints, options include using a low-dose linear model or superlinear model with MOE (if Compound B alkylates "everything," there may be more than one type of damage, and, as cell damage increases, a break and increased slope in the dose-response curve may occur). One group suggested adjusting dose metric for target tissue, considering saturation, cell death, and cell proliferation issues; in addition, high dose excursions may need to be considered. For developmental endpoints, more information is needed (the group hopes to explore this issue at the next colloquium).

- *Additional Data Needs*: The breakout groups differed in opinion regarding the amount of additional information needed to support risk assessment approach decisions. Unanswered questions include: 1) Where are we on the exposure curve (additional low-dose data needed)? 2) Is the parent compound the only bad actor; what about the metabolites? 3) Does glutathione contribute to toxicity? 4) Is detoxification route-specific? 5) At what point is the detoxification mechanism saturated? 6) What is the capacity for repair/cell loss for carcinogenicity versus reproductive capacity versus developmental effects? 7) If Compound B is endogenous, what are sensitive subpopulations (e.g., nutritional aspects)? 8) Are all toxic endpoints covered (has neurotoxicity been explored fully enough)? 9) Were other mechanisms studied (e.g., cell proliferation)? and 10) Is stem cell information available?

  Additional data needs identified by the group include transgenerational/heritable genetic effects data (e.g., low-dose mutation test needed, pre-conceptional exposure data), subchronic exposure data, and exposure duration data for different endpoints.

General Discussions Related to Case Study B

- Discussions focussed on how much weight of evidence and data are needed to support new approaches. The group agreed that extensive research efforts would not be needed for Compound B but that additional low-dose data are needed. The group suggested developing a low-dose model using genetic data as a biomarker of effects; collecting additional data should be easier than conducting chronic animal studies. The group agreed that it is acceptable to move forward even with data gaps, but uncertainties must be recognized and clearly stated. These issues will be explored more closely in the second colloquium.

- While overall consensus was reached that mode of action considerations are appropriate for Compound B, several participants noted that, in the interest of protecting public health and in light of remaining uncertainties, risk managers may want to opt for the most conservative approach (even if that means by-passing mode of action considerations). Linear extrapolation may not be the most conservative approach, and the MOE approach should, therefore, be explored. More data are needed—especially if superlinearity is explored.

- One participant questioned what extra information a risk manager would gain from MOE data. Response: The MOE could tell the risk manager how far away one is from a given exposure scenario, if the risk manager were uncomfortable dealing with anything below the range of observation.

- Another participant questioned how the risk of reproductive effects would be expressed if a low-dose linear model were used (e.g., 10^{-4} to 10^{-6} risk). None of the groups explored this, however.

- Risk assessors/risk managers need to define the desired risk assessment product and consider the following questions: 1) What endpoint will be used to determine "safe" dose? 2) Are all effects adequately characterized? and 3) What effects should be communicated to an exposed public (risk communication)?

  In addition, program needs should be considered; different models may be appropriate for different exposure scenarios.

Case Study C

Group 1 reviewed and evaluated Compound C, a volatile halogenated hydrocarbon with low water solubility. As described in the case study, Compound C, used as an industrial solvent and anesthetic, is a common groundwater contaminant. The case study focussed on kidney and liver toxicity; neurological effects; and carcinogenicity.

The group concluded that toxic effects associated with Compound C (neurological, kidney, and liver toxicity) vary across species and that different modes of action exist for the three endpoints. Metabolism is a critical event in observed toxic responses. Compound C has multiple metabolic pathways, with unmetabolized Compound C excreted via inhalation in a dose-related pattern. Metabolic pathways are qualitatively similar across species (humans, rats, and mice) but quantitatively different, particularly in rats, with mice being the most rapid metabolizers. Both mutagenic and nonmutagenic metabolites are produced, making conclusions regarding mode of action difficult.

Metabolism of Compound C, as summarized below, involves formation of an aldehyde that is reduced to an alcohol, which is either conjugated and eliminated or oxidized to form an acid. A minor pathway involves the formation of a cysteine derivative.

"C" ⇒ CAL ⇒ COH ⇒ CCOOH
⇓
CCYS (minor pathway)

Group 1 treated Compound C as a collection of metabolites and made a matrix that included the different metabolites and their effects, as well as mode of action information. Three major endpoints for humans, rats, and mice are described in the case study: neurotoxicity, liver toxicity, and kidney toxicity, with cross-species concordance seen for liver and neurotoxic effects.

Mode of Action Considerations

Based on its review of available mechanistic data, Group 1 concluded that different modes of action are implicated for the different target sites because different metabolites are involved. In addition, the group concluded that no common mechanism for cancer and noncancer effects appears to exist. The group noted that looking at different dose metrics may be informative. Too many holes exist, however, regarding the metabolites and independent toxicity to form any definitive mode of action conclusions. Because no real site concordance exists, it was suggested that one might ultimately want to develop different risk estimates for kidney and liver effects.

Kidney: The CCYS metabolite may be mutagenic. Evidence of p53 mutation in humans further suggests a genetic mechanism in the kidney.

Liver: The parent compound and the aldehyde and acid are toxic in rodents. The acid is known to be a peroxisome proliferator mediated by a receptor. The aldehyde has been shown to be clastogenic and produce aneuploidy.

Neuro: Neurotoxic response is observed with exposure to the parent, aldehyde, and alcohol. The acid appears to be nontoxic (indicative of binding activity or increased removal). Additional information is needed regarding mode of action.

Dose-Response Implications

The case study provided insufficient quantitative information to allow the group to report on the linearity in the observable range. Based on available data, Group 1 decided that the default approaches are most appropriate—specifically, the linear default for liver and kidney cancer and the RfC/RfD approach for noncancer effects in the kidney. Justification for the linear default for kidney tumors is based on the CCYS mutagenicity and human TS gene mutation. For liver tumors, the group had lower confidence because of limited data on all metabolites and the fact that tumors were seen in mice only. Too many holes in the available data set exist to settle on a nonlinear approach for noncancer effects in the liver. Because mode of action is unclear for neurotoxicity, the group felt it necessary to go with the noncancer default approach.

One participant questioned whether both linear and nonlinear approaches might be considered in light of different actions of the different metabolites.

Data Needs

The group concluded that a closer look at both kidney and liver effects is needed to see whether effects are associated with different metabolites and possibly different modes of action. The group identified the following data needs to enable the full evaluation of common mode of action and low-dose extrapolation:

Data documenting sex and strain of animals for the studies presented.

Additional data on mode of action of individual metabolites.

Additional dose-response data to better define linearity within the test range.

Additional information to evaluate if noncancer effects are associated with cancer effects.
Additional data on the quality and extent of epidemiological data.

Case Study D

Compound D is a water soluble gas absorbed by inhalation and oral routes. It is a major industrial chemical intermediate and a bacterial breakdown product of related compounds in the environment. Nonneoplastic, preneoplastic, and neoplastic changes in the liver were the focus of the case study.

Metabolism is via cytochrome P450, forming an epoxide which is further rearranged to form an aldehyde; both metabolites have electrophilic character. Metabolites are detoxified mainly through GSH conjugation. The case study does not include metabolic data for humans. Effects observed in humans (as reported in several retrospective and prospective cohort studies) include liver cancer and angiosarcomas. Other liver effects include impaired function and

morphological transformations (e.g., hypertrophy/hyperplasia). Liver toxicity is observed in rats, mice, and hamsters exposed to Compound D by oral and inhalation routes. Hepatocellular carcinomas and angiosarcomas were reported in rat dietary studies. Study and dose information for rat studies are detailed in the case study.

Based on the available data, Group 2 concluded that liver toxicity was consistent across species and routes of exposure. The mechanism of action is via mutation of the p53 tumor suppressor gene and *ras* and *myc* oncogenes. The two critical events appear to be metabolism and interaction with DNA, but additional metabolic data in humans is needed to provide a conclusive link. Because a genetic basis of action is assumed, the group recommended the default linear dose-response model. The case study was relatively straightforward, but the group would like assurances that other (non-liver) effects were studied and not found. In addition, insufficient data were available to evaluate whether similar mechanisms were responsible for toxic effects to the liver (structural changes) and liver cancers.

In followup discussions to the case study presentation, several comments were made regarding preneoplastic and nonneoplastic effects. The significance of reported foci in Compound D exposed rats was questioned. It was noted that the foci are of predictive value from a qualitative perspective, but insufficient quantitative data are available to make a full call; foci could be a "jumping-off point" when looking at hepatocellular carcinomas, but not angiosarcomas. Participants questioned whether the linear default should be used for all liver endpoints (assuming that foci are precursors to the cancers) and not consider nonneoplastic effects (i.e., not develop an RfD). The group did not examine this issue, but recommended exploring it at the next colloquium.

One participant noted that time should not be wasted on re-examining mode of action of a known human carcinogen such as Compound D. Another participant noted, however, that further exploration of mode of action issues for a chemical like Compound D may enable a fuller understanding of how tumors originate.

Case Study E

Compound E is described in the case study as a common contaminant found in drinking water, existing in a variety of oxidation states, complexes, and methylated forms. A range of external (skin) and internal toxicities have been shown to be associated with Compound E.

Human data, some *in vitro* data, and little animal data are available for Compound E. Metabolic reduction leads to toxicity, and metabolic pathways are similar in animals and humans. Methylation leads to detoxification, but Compound E may compete with methylation enzymes, affecting DNA methylation at high doses. Noncancer effects include cardiovascular, skin, blood, and liver effects, and pulmonary effects at high oral doses. Critical doses are within the same range for the observed effects, with skin effects seen at the lowest doses. Cancer effects have been observed in humans in skin, bladder, kidney, lung, and possibly other sites and occur largely

via the oral route. Compound E is not an initiator but may serve as a promoter in animals. Most animal tests indicate no noncancer effects.

Compound E is a weak or inactive mutagen, but it does have chromosomal effects (causes breaks in chromosomes); it may be a weak co-mutagen. Group 3 listed the points that led them to their conclusions: 1) liver dysfunction leads to increased skin cancer; 2) Compound E *in vitro* hypermethylates p53; 3) methylated Compound E was a promoter in some initiation/promoter studies in animals; 4) no noncancer effects occur in animals; 5) Compound E can interact with proteins, including effects on energy; and 5) Compound E appears to affect DNA repair and causes oxidative damage.

Group 3 presented the following "highly speculative" hypothesis on the mode of action of Compound E, emphasizing that pieces of information are missing: Compound E acts via methylation of the p53 gene; the methylated form initiates a promotion effect and interacts with the protein, impairing DNA repair, which leads to oxidative damage. Additional data are needed on the hypermethylation of DNA and possible sensitive subpopulations. In addition, Group 3 could not develop a complete linked model concerning the mode of action of Compound E; while interference with DNA repair could be a common mode of action, multiple mechanisms cannot be excluded.

Dose effects are uncertain and the group could not reach consensus on a low-dose model based on available information. The group discussed options, including the default linear model, but also noted that, because of the chromosomal effects caused by Compound E, a linear dose model may not be appropriate. (One nongroup member did comment, however, that the mechanism seen here actually calls for a low-dose linear model.) Sensitive subpopulations must also be considered when developing an appropriate model. *The group commented that this example points to the need for policy, guidance, or a description of data quality objectives in order to move away from defaults.*

The discussions that followed the group presentation addressed the shape of the dose-response curve, common mode of action, and population sensitivity/genetic predisposition issues related to Compound E. One participant commented that the dose-response may be linear at low dose, but, because of complex interactions, may be various shapes at higher doses; learning how to convey this type of scenario to the risk manager is important and challenging.

Unanswered questions stemming from these discussions include:

- What do you do when there are multiple chemicals or environmental justice issues?
- Would we change the model for just one chemical?
- Is it possible to come up with a policy that is protective of all sensitive subpopulations? Also, how do we define the subpopulation (wide amount of variability across the population), especially in light of some sensitivities being induced by multiple chemical exposure?
- If the outcome we are looking at has various causes, how do we deal with the added "noise" of the chemical we may be studying? How do we show that Compound "X" adds to the load (e.g., cardiovascular risk, cancer risk)? How do we factor in genetic susceptibility?

SECTION FIVE

CLOSING PLENARY SESSION

The final session of the colloquium provided participants an opportunity to revisit mode of action and harmonization issues discussed during the previous day and a half and to discuss expectations concerning the second colloquium.

To initiate and guide the closing discussions, the facilitator posed the questions listed below and encouraged participants to look at mode of action and harmonization issues in a broad way.

- Given what is known about the mode of action of various compounds, is there a scientific basis for routinely assuming a different mode of action leading to carcinogenesis and other toxicological effects?

- Mode of action information has been used to influence the approach for low-dose extrapolation. Are there other areas where mode of action information should play a role in risk assessment?

- How do you see mode of action considerations influencing quantitative aspects of risk assessment (e.g., uncertainty factors, dosimetric adjustments, etc.)?

The deliberations that followed covered a variety of related topics, as highlighted in the following sections.

Refining the Definition of Mode of Action

Participants reflected throughout the colloquium on the best way to define mode of action, not only for the purposes of this colloquium but also in the context of risk assessment in general. In the final sessions, the group revisited the definition provided at the opening of the workshop. While this definition did not appear to limit colloquium discussions, some participants challenged the terms “key” biological effects (makes it too narrow), “toxic” responses (how do we define an adverse effect?), and “linked.”

The group offered a number of thoughts on defining mode of action and its role in risk assessment; these are listed below. The overall consensus was that having a working definition at this point in the process is not essential, and that a better definition would likely evolve from discussions such as these. In general, the group decided, mode of action is simply the tool that enables scientists to incorporate more biology into risk assessment and do a better job predicting risks.

- The definition is important, though it is more important at this point to to think about the issues, focussing on metabolism/mechanisms and looking for a simplifying step when sorting through available data.

- Some participants noted that it is important to clearly distinguish between "mode of action" and "mechanism."

- Available empirical data, although not mechanistic, might have some relevance and therefore should be considered when discussing mode of action.

- More useful than the definition is thinking about how Compound X brings about toxic effects and how we describe these events to demonstrate that we understand what is happening at the cellular level. Understanding what "it" is doing to the cell is important, after first defining what "it" is.

- Key biological events are dictated by the data being reviewed. It is important to look at available information on biological events and decide what is key, how well characterized and accurate the events are, and how well links have been developed.

- Key biological events may not be independent but rather a series of events leading to an effect; the concept of sequence is important. This statement was qualified by one participant who noted that a set of conditions may exist which is not necessarily a sequence. Even with the existence of a known sequence, the biological point of departure from a common pathway might not constitute the critical rate-limiting step for a particular observed endpoint. The distinction between the critical event and the many biological conditions that contribute to that event is important.

- Conceptually, we are trying to learn more about certain toxic effects, how effects come about, and if/how knowledge of the mechanism helps us to better predict risk in humans, particularly at low exposure doses. Mode of action is the event or series of events that tells what form the risk model should take.

- The following "framework," developed several years ago when first looking at biologically based models, was offered by one participant as a possible way of thinking about mode of action:

  initial exposure ⇒ delivery to target site ⇒ response ⇒ pathogenesis ⇒ outcome

  Additional steps or pathways may exist, but this provides an overall framework.

- Agreeing on a clear definition of mode of action is important. If we say mode of action is "everything" that happens, it is no longer a meaningful term.

Harmonization Issues

The following bullets summarize a group discussion on the need for more emphasis on the commonality across endpoints.

- The concept of threshold is no longer a useful one in the nonneoplastic arena. As with cancer, the approach for noncancer effects is a function of the type of data historically available. The focus now is on what level is adverse and what is causing it. Framing noncancer effects in terms of threshold is not useful, given the level of detail in contemporary bioassays.

- One participant emphasized that the group needs to "push the envelope" more with respect to looking at mode of action across endpoints. The participant noted that the case studies did not allow the group to do that fully (e.g., if a strong mutagen is being evaluated, let's look closely at the noncancer effects and try to establish a possible common mode of action).

Is There a Scientific Basis for Routinely Assuming a Different Mode of Action Leading to Cancer and Other Toxic Effects?

- No. Consistent with discussions throughout the 2-day colloquium, participants agreed that similar modes of action could be responsible for cancer and noncancer endpoints, but that examples certainly exist where different mechanisms may be responsible for the two endpoints. Also, we cannot assume that all cancers are caused by a single mechanism or that all noncancer effects are caused by one mechansim.

Are There Areas Where Mode of Action Information Should Play a Role in Risk Assessment (Other Than in Influencing Low-Dose Extrapolation Methods)?

Expanding upon the examples provided in earlier plenary sessions (see Section Three), the group provided examples where mode of action should be used in risk assessment to accomplish the following:

- Explore the toxicology of a chemical of interest and evaluate what happens to the cell, at what level adversity is observed, and whether it is a predictive indicator of observed effects.

- Evaluate whether high-dose effects also occur at low doses and if there is route extrapolation.

- Evaluate whether the same effects occur and the same mechanisms are observed in animals and humans.

- Encourage risk assessors to look across a range of endpoints and routes, examining the whole toxicological database. Such an approach allows the risk assessor to strengthen his/her position.

- Enable risk assessors to better determine additive effects for chemicals with common modes of action.

- Evaluate multiple routes of exposure within an animal or human to the same chemical (aggregate risk).

- Define a common surrogate for dose and response. The tobacco-specific mutagen NNK is a good example of this; NNK produces cancer in animals and the dose-response curves for NNK and its associated adducts can be overlapped. As a result, the dose-response curve can be extended to doses below which tumors can be measured because the surrogate (i.e., the adducts) is a more sensitive measure of dose. Caution needs to be taken, however, if the chosen surrogate is not the "critical" or "limiting" factor; some measure of the efficiency of the endpoint would therefore be needed, which comes back to the issue of linking the precursor to the adverse effect. In many cases, depending on the questions being asked, surrogates for dose and response may be different: a good surrogate for the dose might identify the biological point of departure, while a good surrogate for the response may reflect the critical event linked to that effect. Ideally, a series of dose-response curves for the endpoints and for the surrogate should be developed.

- Improve the inferences made from structural activity relationships for untested chemicals.

- Evaluate "residual risk" (i.e., what does an exceedance of a "safe" dose really mean?). For example, in a case where an RfD is exceeded by 10 times, looking at mode of action of the chemical enables a further evaluation of public health significance.

- Develop new test methodologies.

In summary, use of mode of action should improve the risk assessment process, enabling us to develop new approaches based on the new science. Developing a useful approach to evaluating mode of action issues when multichemical/multimedia exposures exist is necessary to meet regional risk assessor needs.

How Do Mode of Action Considerations Influence Quantitative Aspects of Risk Assessment?

The group agreed that the examples listed in the preceding section have quantitative relevance. Studying the best way(s) to incorporate these concepts into the quantitative risk assessment is the next step in the process.

Looking Ahead to Colloquium #2

The group expressed interest in and enthusiasm about the upcoming second colloquium, where they will evaluate the more quantitative aspects of mode of action, testing some of the hypotheses discussed during the first colloquium. Participants offered suggestions on the best way to approach the second colloquium. Of particular interest was the prospect of performing a full quantitative exercise using Case Study B (e.g., dose response on adducts). Participants noted that it may be worthwhile to look more closely at real-life examples where mode of action considerations made a large difference in the risk assessment (e.g., alpha-2μ globulin, thyroid tumors, bladder tumors).

Participants agreed to further contemplate approaches for the next colloquium and provide their suggestions to the planning committee.